PATENT
016303-0024300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Jeffery J. Wheeler *et al.*

Application No.: 09/431,594

Filed: November 1, 1999

For: LIPID-NUCLEIC ACID PARTICLES PREPARED VIA HYDROPHOBIC LIPID-NUCLEIC ACID COMPLEX INTERMEDIATE AND USE FOR GENE TRANSFER

Customer No.: 20350

Confirmation No. 8936

Examiner: Janet L. Epps

Art Unit: 1635

Declaration of Sean C. Semple, M.Sc. Under 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Sean C. Semple, M.Sc., state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently a Senior Scientist at Inex Pharmaceuticals Corporation (Burnaby, Canada), a biotechnology company whose primary focus is the development of cancer treatments that are based on its propriety drug delivery platform and that are more effective and have fewer side effects than conventional cancer treatments. I have been employed at Inex Pharmaceuticals Corporation since 1994. I have been a Senior Scientist at Inex Pharmaceuticals Corporation since 2001.

3. In 1991, I graduated from the University of British Columbia, with a Bachelor of Science, Honours degree in Biochemistry. In 1994, I was awarded my M. Sc. in Biochemistry and Molecular Biology from the University of British Columbia. My graduate studies were carried out under the direction of P.R. Cullis, a Professor in the Department of Biochemistry,

University of British Columbia, and my dissertation was entitled "Factors influencing the activation of plasma proteins that interact with liposomes *in vivo*."

4. Attached hereto as Exhibit A is a true copy of my *curriculum vitae* and a list of publications of which I am an author or co-author.

5. I have read and am familiar with the contents of the above-referenced patent application. In addition, I have read the Final Office Action, mailed March 25, 2003, received from the United States Patent & Trademark Office in the above-referenced patent application. It is my understanding that the Examiner is concerned that claims 42 and 44-75 are anticipated under 35 U.S.C. § 102(e) over U.S. Patent No. 5,820,873 ("Choi *et al.*"). For the reasons set forth herein, the Examiner's concern is overcome.

6. The presently claimed invention is directed, *inter alia*, to nucleic acid-lipid particles for introducing a nucleic acid into a cell either *in vitro* or *in vivo*. More particularly, independent claim 42 reads as follows:

> 42. (Amended) A nucleic acid-lipid particle for introducing a nucleic acid into a cell, the particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid in said particle is resistant in aqueous solution to degradation with a nuclease. method of introducing a nucleic acid into a cell, the nucleic acid being serum-stable and protected from degradation.

7. The specification teaches methods of making lipid-nucleic acid particles via novel, hydrophobic nucleic acid-lipid intermediate complexes. Manipulation of these complexes in either detergent-based or organic solvent-based systems leads to nucleic acid-lipid particles, wherein the nucleic acid in the nucleic acid-lipid particles is protected from nuclease degradation.

8. It is my understanding that Choi *et al.* is cited by the Examiner as allegedly disclosing particles that meet the structural limitations of the particles produced by the methods of the instant invention (*see*, page 3 of the Office Action).

9. I have reviewed the Choi *et al.* patent, and it is my opinion that Choi *et al.* do ***not*** teach (or even suggest) a nucleic acid-lipid particle, wherein the nucleic acid in the nucleic acid-

lipid particle is resistant in aqueous solution to degradation with a nuclease as is recited in claim 42 and, in turn, dependent claims 44-75.

10. In fact, a perusal of Choi *et al.* reveals that in the Examples set forth therein methods for loading therapeutic agents, *e.g.*, vincristine, into liposomes are disclosed. More particularly, Example 9 sets forth the following loading (or encapsulation) method:

> The dry lipid was hydrated with 300 mM citrate buffer, pH 4.0. Following extrusion, the vesicles (100 mg/mL) were added to a solution of vincristine (Oncovin; 1 mg/ml) to achieve a drug:lipid ration of 0.1:1. The exterior pH of the liposome/vincristine mixture was raised to pH 7.0-7.2 by titration with 500 mM sodium phosphate and immediately the sample was heated to 60°C for 10 minutes to achieve encapsulation of the vincristine.

See, Example 9, column 21, lines 11-18. Example 10 sets forth a similar loading/encapsulation procedure for loading vincristine into liposomes (*see*, Example 10, column 21, line 62 through column 22, line 9).

11. The loading/encapsulation methods disclosed in Choi *et al.* are useful for loading small molecules (*e.g.*, vinca alkaloids, *etc.*) into liposomes, but are not useful for loading nucleic acids (*e.g.*, oligonucleotides, plasmid DNA, *etc.*) into liposomes because nucleic acids do ***not*** readily cross intact lipid membranes. As such, if one were to use the loading/encapsulation methods disclosed in Choi *et al.* and were to add external plasmid DNA to preformed liposomes in aqueous buffer, one would not expect to see any entrapment of the plasmid DNA in the liposomes. Again, this is because nucleic acids do not readily cross intact lipid membranes.

12. As of the filing date of the Choi *et al.* patent, *i.e.*, 1994-1995, the state-of-the-art was to prepare cationic liposomes and, then, to complex the preformed cationic liposomes with DNA in an aqueous solution to form DNA-cationic liposome complexes (*i.e.*, lipoplexes). Given that DNA does not readily cross lipid membranes and that the cationic lipids present in the external membrane of the vesicles would electrostatically interact with the negatively charged DNA, the mixing of DNA with preformed cationic liposomes in aqueous solution does ***not*** result in entrapment of DNA within the internal, aqueous space of the liposomes. Moreover, the lipoplexes formed by this proves are ill-defined, are only partially protected from nucleases, are heterogeneous in size and are rapidly cleared

from the circulation (*see*, Figure 2 of Wheeler *et al.*, *Gene Therapy*, *6*:271-281 (1999); and Figure 1 of Monck *et al.*, *J. Drug Targeting*, *7(6)*:439-452 (2000), copies of which are attached hereto as Exhibits B and C).

13. As such, when Choi *et al.* state that cationic carriers of DNA can be improved through the addition of PEG lipids, such as the PEG-ceramide conjugates disclosed and claimed therein, Choi *et al.* are referring to the preformed cationic liposome carriers that are then complexed with DNA to form lipoplexes as described above. In fact, the examples provided in Choi *et al.* that are directed to such preformed cationic liposomes demonstrate that aggregation of the cationic liposomes alone (no DNA) can be inhibited in the presence of serum (most serum proteins carry a net negative charge) if the liposomes contain a PEG-ceramide conjugate.

14. In contrast to the teachings of Choi *et al.*, the present invention provides novel methods by which nucleic acids (*e.g.*, oligonucleotides, plasmid DNA, *etc.*) are entrapped, *i.e.*, encapsulated, within individual cationic liposomes that include a conjugated lipid, such as a PEG-lipid conjugate. As explained in the specification and as set forth in the presently pending claims, the PEG-lipid conjugate prevents aggregation of the particles during formation, thereby resulting in nucleic acid-lipid particles of a homogeneous and defined size containing nucleic acid that is fully encapsulated in the lipid bilayer such that the nucleic acid is completely protected from nuclease degradation. This is in stark contrast to the lipoplexes that would be formed based on the cationic liposomes of Choi *et al.*, which contain PEG-ceramide conjugates.

15. In view of the foregoing, it is my opinion that the Choi *et al.* patent does ***not*** teach (or even suggest) the nucleic acid-lipid particles recited in claims 42 and 44-75 because Choi *et al.* do ***not*** teach (or even suggest) (1) nucleic acid-lipid particles, wherein the nucleic acid in the nucleic acid-lipid particles is resistant in aqueous solution to degradation with a nuclease, or (2) methods for making such nucleic acid-lipid particles.

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Sept. 24, 2003

Sean C. Semple, M.S.

60042843 v1